Microfluidic Biochip Design

Abstract

As humans prepare for the exploration of our solar system, there is a growing need for miniaturized medical and environmental diagnostic devices for use on spacecrafts, especially during long-duration space missions where size and power requirements are critical. In recent years, the biochip (or Lab-on-a-Chip) has emerged as a technology that might be able to satisfy this need. In generic terms, a biochip is a miniaturized microfluidic device analogous to the electronic microchip that ushered in the digital age. It consists of tiny microfluidic channels, pumps and valves that transport small amounts of sample fluids to biosensors that can perform a variety of tests on those fluids in near real time. It has the obvious advantages of being small, lightweight, requiring less sample fluids and reagents and being more sensitive and efficient than larger devices currently in use. Some of the desired space-based applications would be to provide smaller, more robust devices for analyzing blood, saliva and urine and for testing water and food supplies for the presence of harmful contaminants and microorganisms. Our group has undertaken the goal of adapting as well as improving upon current biochip technology for use in long-duration microgravity environments.

In addition to developing computational models of the microfluidic channels, valves and pumps that form the basis of every biochip, we are also trying to identify potential problems that could arise in reduced gravity and develop solutions to these problems. One such problem is due to the prevalence of bubbly sample fluids in microgravity. A bubble trapped in a microfluidic channel could be detrimental to the operation of a biochip. Therefore, the process of bubble formation in microgravity needs to be studied, and a model of this process has been developed and used to understand how bubbles develop and move through biochip components. It is clear that some type of bubble filter would be necessary in Space, and several bubble filter designs are being evaluated.

Another area of current research is DNA hybridization on solid surfaces. In DNA hybridization, short single strands of DNA, called oligonucleotides (oligos), are bonded to a solid substrate such as glass, and then fluid containing unknown DNA flows over it. The bound DNA are called the probes, and the suspended DNA are called the targets. A fluorescent molecule is attached to each target DNA. If the target and probe DNA complement each other, they will hybridize (zip up), and then the fluorescent molecules will emit detectable light when stimulated by a laser. The intensity of the fluorescent signal relates to the degree of surface hybridization and can be used as a means of identifying the unknown DNA. This is the basis of many commercial DNA microarrays such as the GeneChip made by Affymetrix, which can contain hundreds of thousands of oligo spots and are used primarily for studying gene expression.

Another application of this technique currently under consideration is the identification of

microorganisms from their unique genetic signature. This is particularly important to NASA because such a device could be used to test for contaminated food and water supplies onboard distant spacecraft or outposts. The basic principle would be to deposit an array of oligo spots that uniquely identify the organisms most commonly suspected for contamination. Then, the process of surface hybridization could be used to see if any of the DNA from the unknown samples match the known DNA. The exact details of this process have not yet been completely worked out, but a number of companies such as HealthSpex are working on prototype devices.

There are a number of problems that need to be solved before this process becomes practical. First, the list of desired microorganisms needs to be determined, and from the genome of every organism on that list, short DNA sequences (oligos) need to be selected that are capable of uniquely identifying them. The DNA from the sample needs to be extracted, purified and possibly amplified before passing over the immobilized probes. In order to make the device as small as possible, it would be desirable to integrate the sample preparation, transport and detection processes into a single miniaturized device. In the ideal device, microfluidic channels would transport small amounts of sample fluid over the probe sites, and optical sensors integrated directly into the microchip would detect the hybridization.

Current research efforts are focusing on the optimization of this type of biosensor. The critical question is whether the fluorescent signal itself can provide enough information to uniquely identify the unknown organisms. This depends first and foremost on the proper choice of the oligo probes. They must be unique enough to differentiate the unknown organism from any other organism. This depends on where in the genome the oligo is selected and how long it is. It is generally believed that a length of about 25-30 nucleotide pairs is a good choice for gene expression analysis, but it is not clear that this would be the optimal length for microbial identification.

One problem that arises is how to distinguish between perfect complementary hybridization and single-base-pair mismatch hybridization. Without this ability, it would be impossible to differentiate between closely related organisms. One way of alleviating this problem is to apply a temperature gradient along the substrate and exploit changes in the kinetics of the hybridization process with temperature. Each complementary oligo pair has a unique melting temperature (the temperature at which half the available oligos are paired), and the melting temperature between a perfect match and a single mismatch can often be as large as 10 degrees Celsius. The melting temperature depends on the composition of the oligo as well as its length. If the position of the oligo spot on the substrate corresponds to its melting temperature, then different surface hybridization patterns can be used to distinguish between complementary and non-complementary duplexes.

In order to examine all of these issues, a computational model of DNA hybridization in a

microchannel has been developed. Assuming the kinetics of the hybridization process are known, it can be used to optimize the design of the detector to improve its sensitivity and specificity for the set of microorganisms under consideration. In addition to the choice of oligo, other factors that can be varied include the channel dimensions, flow rate, inlet concentrations, sensor area and temperature profile. This model is used to predict the degree of hybridization and the unique surface concentration pattern for each case. In order to help with the problem of oligo selection, software has been developed that can compute the thermodynamic properties of the oligos such as their entropy, enthalpy, Gibbs free energy and melting temperature from known empirical and statistical data. It is also able to calculate the changes in these properties due to mismatches. This software is integrated directly into the model.